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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :		(11) International Publication Number: WO 98/46641
C07K 14/475, 14/435, 14/47, C12N 15/12, 15/18, 5/10, 15/63, 15/64, 15/65, 15/66	A1	(43) International Publication Date: 22 October 1998 (22.10.98)

(21) International Application Number: PCT/US98/07603

(22) International Filing Date: 15 April 1998 (15.04.98)

(30) Priority Data:
08/843,651 16 April 1997 (16.04.97) US

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(81) Designated States: AU, CA, JP, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: NOVEL POLYPEPTIDES WITHIN THE GROWTH FACTOR SUPERFAMILY

(57) Abstract

The invention relates to Tango-67 polypeptides, nucleic acid molecules encoding Tango-67, and uses thereof. Tango-67 is related to a number of growth factors, particularly members of the connective tissue growth factor family.

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NOVEL POLYPEPTIDES WITHIN THE GROWTH FACTOR SUPERFAMILY

Background of the Invention

The size and differentiated characteristics of cellular compartments are controlled in part by the availability of extracellular growth factors. These growth factors can influence cellular replication, cell survival, as well as the function of differentiated end cells. The ability to control the expansion of specific cell types *in vivo* has demonstrated clinical utility, the best examples being the stimulation of red and white blood cell production by erythropoietin and granulocyte colony stimulating factor, respectively. The utility of growth factors for the treatment of other human disorders (e.g., neurodegeneration) is currently being examined, and it is hoped that in certain instances providing exogenous growth stimuli may arrest or reverse the course of degenerative disorders, or provide for more rapid restoration of function in cases of acute tissue damage (e.g. wound healing).

Secreted growth factors also play an important role in early development. Although the details of this process are incompletely understood and vary considerably from species to species, genetic analysis in model organisms has demonstrated an important role for growth factors in the differentiation of the early embryo. One such molecule is the product of the *twisted gastrulation* gene (TSG), mutations in which lead to defects in embryogenesis. TSG messenger RNA (mRNA) is present in the early embryo and is important for specification of cell fates along the dorsal midline. TSG has been molecularly characterized, and is a cysteine-rich secreted protein that shows homology to connective tissue growth factor (CTGF). CTGF is itself a mitogen for

fibroblasts and shares antigenic determinants with platelet derived growth factor (PDGF).

Summary of the Invention

The invention relates to the discovery and characterization of Tango-67, a new soluble growth factor. A form of Tango-67 described herein is a 224 amino acid, cysteine rich polypeptide. Northern blot analysis of Tango-67 mRNA reveals that it is present at varying levels in a wide variety of tissues.

The invention features an isolated nucleic acid molecule encoding a Tango-67 polypeptide. For example, the isolated nucleic acid molecule encodes the secreted form of human Tango-67, or a polypeptide having sequence that is at least 85% identical to the sequence of SEQ ID NO:2. In other embodiments, the isolated nucleic acid molecule claim 1 includes a nucleotide sequence encoding the amino acid sequence of SEQ ID NO:2; and includes the nucleotide sequence of between nucleotide 182 and 850, inclusive, of SEQ ID NO:1.

In other embodiments, the isolated nucleic acid molecule encoding Tango-67 hybridizes to a nucleic acid molecule having the sequence of nucleotides 182 to 850, inclusive, of SEQ ID NO:1 or its complement. In other embodiments, the hybridization occurs under stringent conditions.

In other embodiments the isolated nucleic acid molecule encoding Tango-67 encodes a Tango-67 polypeptide capable of promoting cell proliferation and/or differentiation.

The invention also features a host cell which includes and isolated nucleic acid molecule encoding Tango-67, a nucleic acid vector (e.g., an expression vector, a vector which includes a regulatory element, a vector which includes a regulatory element selected from

the group consisting of the cytomegalovirus hCMV immediate early gene, the early promoter of SV40 adenovirus, the late promoter of SV40 adenovirus, the lac system, the trp system, the TAC system, the TRC system, the major operator and promoter regions of phage λ, the control regions of fd coat protein, the promoter for 3-phosphoglycerate kinase, the promoters of acid phosphatase, and the promoters of the yeast α-mating factors, vector which includes a regulatory element which directs tissue-specific expression, a vector which includes a reporter gene, a vector which includes a reporter gene selected from the group selected from the group consisting of β-lactamase, chloramphenicol acetyltransferase (CAT), adenosine deaminase (ADA), aminoglycoside phosphotransferase (neo^r, $G418^r$), dihydrofolate reductase (DHFR), hygromycin-B-phosphotransferase (HPH), thymidine kinase (TK), lacZ (encoding β-galactosidase), and xanthine guanine phosphoribosyltransferase (XGPRT), a vector that is a plasmid, a vector that is a virus, a vector that is a retrovirus.

In another embodiment, the invention features a substantially pure Tango-67 polypeptide (e.g., a Tango-67 polypeptide that is soluble under physiological conditions, a Tango-67 polypeptide which includes a signal sequence, a Tango-67 polypeptide that stimulated proliferation and/or differentiation of a cell, a Tango-67 polypeptide that includes an amino acid sequence that is at least 85% identical to the amino acid sequence of SEQ ID NO:2, a Tango-67 polypeptide that includes an amino acid sequence that is at least 90% identical to the amino acid sequence of of SEQ ID NO:2, a Tango-67 polypeptide that includes an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO:2, and a Tango-67 polypeptide that includes an

amino acid sequence that is identical to the amino acid sequence of SEQ ID NO:2.

In other embodiments, the invention features a substantially pure polypeptide which includes a first portion and a second portion, the first portion including a Tango-67 polypeptide and the second portion including a detectable marker.

The invention also features an antibody that selectively binds to a Tango-67 polypeptide (e.g., a monoclonal antibody).

The invention also features a pharmaceutical composition which includes a Tango-67 polypeptide.

Also included in the invention are: a method for detecting Tango-67 in a sample, the method including:

(a) obtaining a biological sample;

(b) contacting the biological sample with an antibody that specifically binds Tango-67 under conditions that allow the formation of Tango-67-antibody complexes; and

(c) detecting the complexes, if any, as an indication of the presence of Tango-67 in the sample.

In another aspect, the invention features a method of identifying a compound that modulates the expression of Tango-67, the method including comparing the level of expression of Tango-67 in a cell in the presence and absence of a selected compound, wherein a difference in the level of expression in the presence and absence of the selected compound indicates that the selected compound modulates the expression of Tango-67.

In another aspect, the invention features, a method of identifying a compound that modulates the activity of Tango-67, the method including comparing the level of activity of Tango-67 in a cell in the presence and absence of a selected compound, wherein a difference in the level of activity in the presence and absence of the selected compound indicates that the selected compound modulates the activity of Tango-67.

In another aspect, the invention features a method for treating a patient suffering from a disorder associated with excessive expression or activity of Tango-67, the method including administering to the patient a compound which inhibits expression or activity of Tango-67.

The invention also features a method for treating a patient suffering from a disorder associated with insufficient expression or activity of Tango-67, the method including administering to the patient a compound which increases expression or activity of Tango-67.

The invention also features a method for diagnosing a disorder associated with aberrant expression of Tango-67, the method including obtaining a biological sample from a patient and measuring Tango-67 expression in the biological sample, wherein increased or decreased Tango-67 expression in the biological sample compared to a control indicates that the patient suffers from a disorder associated with aberrant expression of Tango-67.

In another aspect the invention features a method for diagnosing a disorder associated with aberrant activity of Tango-67, the method including obtaining a biological sample from a patient and measuring Tango-67 activity in the biological sample, wherein increased or decreased Tango-67 activity in the biological sample compared to a control indicates that the patient suffers

from a disorder associated with aberrant activity of Tango-67.

The invention encompasses isolated nucleic acid molecules encoding Tango-67 or a fragment thereof, vectors containing these nucleic acid molecules, cells harboring recombined DNA encoding Tango-67, fusion proteins which include Tango-67, transgenic animals which express Tango-67, recombinant knock-out animals which fail to express Tango-67. Especially preferred are nucleic acid molecules encoding the polypeptide shown in the Figure (SEQ ID NO:2).

The invention encompasses nucleic acids that have a sequence that is substantially identical to a Tango-67 nucleic acid sequence. A nucleic acid which is substantially identical to a given reference nucleic acid molecule is hereby defined as a nucleic acid having a sequence that has at least 85%, preferably 90%, and more preferably 95%, 98%, 99% or more identity to the sequence of the given reference nucleic acid molecule, e.g., the nucleic acid sequence of SEQ ID NO:1.

A polypeptide which is "substantially identical" to a given reference polypeptide molecule is hereby defined as a polypeptide having a sequence that has at least 85%, preferably 90%, and more preferably 95%, 98%, 99% or more identity to the sequence of the given reference polypeptide sequence, e.g., the polypeptide sequence of SEQ ID NO:2.

The nucleic acid molecules of the invention can be inserted into transcription and/or translation vectors, as described below, which will facilitate expression of the insert. The nucleic acid molecules and the polypeptides they encode can be used directly as diagnostic or therapeutic agents, or (in the case of a polypeptide) can be used to generate antibodies that, in turn, are therapeutically useful. Accordingly,

expression vectors containing the nucleic acid molecules of the invention, cells transfected with these vectors, the polypeptides expressed, and antibodies generated, against either the entire polypeptide or an antigenic fragment thereof, are among the preferred embodiments.

A transfected cell is any cell into which (or into an ancestor of which) has been introduced, by means of recombinant DNA techniques, a nucleic acid encoding a polypeptide of the invention (e.g., a Tango-67 polypeptide). An isolated nucleic acid molecule is a nucleic acid molecule that is separated from the 5' and 3' coding sequences with which it is immediately contiguous in the naturally occurring genome of an organism. Isolated nucleic acid molecule include nucleic acid molecule which are not naturally occurring, e.g., nucleic acid molecules created by recombinant DNA techniques.

The term "nucleic acid molecule" encompasses both RNA and DNA, including cDNA, genomic DNA, and synthetic (e.g., chemically synthesized) DNA. Where single-stranded, the nucleic acid may be a sense strand or an antisense strand.

The invention also encompasses nucleic acid molecules that hybridize, preferably under stringent conditions, to a nucleic acid molecule encoding a Tango-67 polypeptide (e.g., a nucleic acid molecule having the sequence shown in SEQ ID NO:1 or a nucleic acid molecule having the sequence of the Tango-67 encoding portion of the sequence of SEQ ID NO:1). Preferably the hybridizing nucleic acid molecule consists of 400, more preferably 200 nucleotides.

Preferred hybridizing nucleic acid molecules have an activity possessed by Tango-67, e.g., the ability to increase proliferation and/or differentiation of cells.

The invention also features substantially pure or isolated Tango-67 polypeptides, including those that correspond to various functional domains of Tango-67, or fragments thereof. The polypeptides of the invention encompass amino acid sequences that are substantially identical to the amino acid sequence shown in the Figure (SEQ ID NO:2).

The polypeptides of the invention can also be chemically synthesized, or they can be purified from tissues in which they are naturally expressed, according to standard biochemical methods of purification.

Also included in the invention are "functional polypeptides," which possess one or more of the biological functions or activities of Tango-67. These functions include the ability to bind some or all of the proteins which normally bind to Tango-67. A functional polypeptide is also considered within the scope of the invention if it serves as an antigen for production of antibodies that specifically bind to Tango-67. In many cases, functional polypeptides retain one or more domains present in the naturally-occurring form of the polypeptide.

The functional polypeptides may contain a primary amino acid sequence that has been modified from those disclosed herein. Preferably these modifications consist of conservative amino acid substitutions, as described herein.

The terms "protein" and "polypeptide" are used herein to describe any chain of amino acids, regardless of length or post-translational modification (for example, glycosylation or phosphorylation). Thus, the term "Tango-67 polypeptides" includes full-length, naturally occurring Tango-67 protein (with or without a signal sequence), as well a recombinantly or synthetically produced polypeptide that correspond to a

full-length naturally occurring Tango-67 protein or to particular domains or portions of a naturally occurring protein. The term also encompasses mature Tango-67 which has an added amino-terminal methionine (useful for expression in prokaryotic cells).

The term "purified" as used herein refers to a nucleic acid or peptide that is substantially free of cellular material, viral material, or culture medium when produced by recombinant DNA techniques, or chemical precursors or other chemicals when chemically synthesized.

Polypeptides or other compounds of interest are said to be "substantially pure" when they are within preparations that are at least 60% by weight (dry weight) the compound of interest. Preferably, the preparation is at least 75%, more preferably at least 90%, and most preferably at least 99%, by weight the compound of interest. Purity can be measured by any appropriate standard method, for example, by column chromatography, polyacrylamide gel electrophoresis, or HPLC analysis.

A polypeptide or nucleic acid molecule is "substantially identical" to a reference polypeptide or nucleic acid molecule if it has a sequence that is at least 85%, preferably at least 90%, and more preferably at least 95%, 98%, or 99% identical to the sequence of the reference polypeptide or nucleic acid molecule.

Where a particular polypeptide is said to have a specific percent identity to a reference polypeptide of a defined length, the percent identity is relative to the reference peptide. Thus, a peptide that is 50% identical to a reference polypeptide that is 100 amino acids long can be a 50 amino acid polypeptide that is completely identical to a 50 amino acid long portion of the reference polypeptide. It might also be a 100 amino acid long polypeptide which is 50% identical to the reference

polypeptide over its entire length. Of course, many other polypeptides will meet the same criteria.

In the case of polypeptide sequences which are less than 100% identical to a reference sequence, the non-identical positions are preferably, but not necessarily, conservative substitutions for the reference sequence. Conservative substitutions typically include substitutions within the following groups: glycine and alanine; valine, isoleucine, and leucine; aspartic acid and glutamic acid; asparagine and glutamine; serine and threonine; lysine and arginine; and phenylalanine and tyrosine.

For polypeptides, the length of the reference polypeptide sequence will generally be at least 16 amino acids, preferably at least 20 amino acids, more preferably at least 25 amino acids, and most preferably 35 amino acids, 50 amino acids, or 100 amino acids. For nucleic acids, the length of the reference nucleic acid sequence will generally be at least 50 nucleotides, preferably at least 60 nucleotides, more preferably at least 75 nucleotides, and most preferably 100 nucleotides or 300 nucleotides.

Sequence identity can be measured using sequence analysis software (for example, the Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, WI 53705), with the default parameters as specified therein.

The nucleic acid molecules of the invention can be inserted into a vector, as described below, which will facilitate expression of the insert. The nucleic acid molecules and the polypeptides they encode can be used directly as diagnostic or therapeutic agents, or can be used (directly in the case of the polypeptide or indirectly in the case of a nucleic acid molecule) to

generate antibodies that, in turn, are clinically useful as a therapeutic or diagnostic agent. Accordingly, vectors containing the nucleic acid of the invention, cells transfected with these vectors, the polypeptides expressed, and antibodies generated, against either the entire polypeptide or an antigenic fragment thereof, are among the preferred embodiments.

As used herein, the term "transformed cell" means a cell into which (or into an ancestor of which) has been introduced, by means of recombinant DNA techniques, a nucleic acid molecule encoding a polypeptide of the invention.

The invention also features antibodies, e.g., monoclonal, polyclonal, and engineered antibodies, which specifically bind Tango-67. By "specifically binds" is meant an antibody that recognizes and binds to a particular antigen, e.g., the Tango-67 polypeptide of the invention, but which does not substantially recognize or bind to other molecules in a sample, e.g., a biological sample, which includes Tango-67.

The invention also features antagonists and agonists of Tango-67 that can inhibit or enhance one or more of the functions or activities of Tango-67, respectively. Suitable antagonists can include small molecules (i.e., molecules with a molecular weight below about 500), large molecules (i.e., molecules with a molecular weight above about 500), antibodies that bind and "neutralize" Tango-67 (as described below), polypeptides which compete with a native form of Tango-67 for binding to a protein, e.g., the Tango-67 receptor, and nucleic acid molecules that interfere with transcription of Tango-67 (for example, antisense nucleic acid molecules and ribozymes). Agonists of Tango-67 also include small and large molecules, and antibodies other than "neutralizing" antibodies.

The invention also features molecules which can increase or decrease the expression of Tango-67 (e.g., by influencing transcription or translation). Small molecules (i.e., molecules with a molecular weight below about 500), large molecules (i.e., molecules with a molecular weight above about 500), and nucleic acid molecules that can be used to inhibit the expression of Tango-67 (for example, antisense and ribozyme molecules) or to enhance their expression (for example, expression constructs that place nucleic acid sequences encoding Tango-67 under the control of a strong promoter system), and transgenic animals that express a Tango-67 transgene.

In addition, the invention features substantially pure polypeptides that functionally interact with Tango-67, e.g., a Tango-67 receptor, and the nucleic acid molecules that encode them.

The invention encompasses methods for treating disorders associated with aberrant expression or activity of Tango-67. Thus, the invention includes methods for treating disorders associated with excessive expression or activity of Tango-67. Such methods entail administering a compound which decreases the expression or activity of Tango-67. The invention also includes methods for treating disorders associated with insufficient expression of Tango-67. These methods entail administering a compound which increases the expression or activity of Tango-67.

The invention also features methods for detecting a Tango-67 polypeptide. Such methods include: obtaining a biological sample; contacting the sample with an antibody that specifically binds Tango-67 under conditions which permit specific binding; and detecting any antibody-Tango-67 complexes formed.

In addition, the present invention encompasses methods and compositions for the diagnostic evaluation,

typing, and prognosis of disorders associated with inappropriate expression or activity of Tango-67. For example, the nucleic acid molecules of the invention can be used as diagnostic hybridization probes to detect, for example, inappropriate expression of Tango-67 or mutations in the Tango-67 gene. Such methods may be used to classify cells by the level of Tango-67 expression.

Alternatively, the nucleic acid molecules can be used as primers for diagnostic PCR analysis for the identification of gene mutations, allelic variations and regulatory defects in the Tango-67 gene. The present invention further provides for diagnostic kits for the practice of such methods.

The invention features methods of identifying compounds that modulate the expression or activity of Tango-67 by assessing the expression or activity of Tango-67 in the presence and absence of a selected compound. A difference in the level of expression or activity of Tango-67 in the presence and absence of the selected compound indicates that the selected compound is capable of modulating expression or activity or Tango-67. Expression can be assessed either at the level of gene expression (e.g., by measuring mRNA) or protein expression by techniques that are well known to skilled artisans. The activity of Tango-67 can be assessed functionally, i.e., by assaying the ability of the compound to inhibit proliferation of myeloid cells.

The preferred methods and materials are described below in examples which are meant to illustrate, not limit, the invention. Skilled artisans will recognize methods and materials that are similar or equivalent to those described herein, and that can be used in the practice or testing of the present invention.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as

commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are described herein. In addition, the materials, methods, and examples are illustrative only and are not intended to be limiting.

Other features and advantages of the invention will be apparent from the detailed description, and from the claims.

Brief Description of the Drawing

The Figure is a depiction of the nucleotide sequence encoding Tango-67 and 3' and 5' non-translated sequence (SEQ ID NO:1) and the amino acid sequence (SEQ ID NO:2) of Tango-67.

Detailed Description

Tango-67 is a new member of the growth factor superfamily. At the protein sequence level, Tango-67 is related to the product of the drosophila twisted gastrulation gene and human connective tissue growth factor.

Tango-67 Nucleic Acid Molecules

The Tango-67 nucleic acid molecules of the invention can be cDNA, genomic DNA, synthetic DNA, or RNA, and can be double-stranded or single-stranded (*i.e.*, either a sense or an antisense strand). Fragments of these molecules are also considered within the scope of the invention, and can be produced, for example, by the polymerase chain reaction (PCR) or generated by treatment with one or more restriction endonucleases. A ribonucleic acid (RNA) molecule can be produced by

in vitro transcription. Preferably, the nucleic acid molecules encode polypeptides that, regardless of length, are soluble under normal physiological conditions.

The nucleic acid molecules of the invention can contain naturally occurring sequences, or sequences that differ from those that occur naturally, but, due to the degeneracy of the genetic code, encode the same polypeptide (for example, the polypeptide of SEQ ID NO:2). In addition, these nucleic acid molecules are not limited to sequences that only encode polypeptides, and thus, can include some or all of the non-coding sequences that lie upstream or downstream from a coding sequence.

The nucleic acid molecules of the invention can be synthesized (for example, by phosphoramidite-based synthesis) or obtained from a biological cell, such as the cell of a mammal. Thus, the nucleic acids can be those of a human, mouse, rat, guinea pig, cow, sheep, horse, pig, rabbit, monkey, dog, or cat. Combinations or modifications of the nucleotides within these types of nucleic acids are also encompassed.

In addition, the isolated nucleic acid molecules of the invention encompass fragments that are not found as such in the natural state. Thus, the invention encompasses recombinant molecules, such as those in which a nucleic acid molecule (for example, an isolated nucleic acid molecule encoding Tango-67) is incorporated into a vector (for example, a plasmid or viral vector) or into the genome of a heterologous cell (or the genome of a homologous cell, at a position other than the natural chromosomal location). Recombinant nucleic acid molecules and uses therefor are discussed further below.

In the event the nucleic acid molecules of the invention encode or act as antisense molecules, they can be used for example, to regulate translation of Tango-67.

Techniques associated with detection or regulation of Tango-67 expression are well known to skilled artisans and can be used to diagnose and/or treat inflammation or disorders associated with cellular proliferation. These nucleic acid molecules are discussed further below in the context of their clinical utility.

The invention also encompasses nucleic acid molecules that hybridize under stringent conditions to a nucleic acid molecule encoding a Tango-67 polypeptide. The cDNA sequence described herein (SEQ ID NO:1) can be used to identify these nucleic acids, which include, for example, nucleic acids that encode homologous polypeptides in other species, and splice variants of the Tango-67 gene in humans or other mammals. Accordingly, the invention features methods of detecting and isolating these nucleic acid molecules. Using these methods, a sample (for example, a nucleic acid library, such as a cDNA or genomic library) is contacted (or "screened") with a Tango-67-specific probe (for example, a fragment of SEQ ID NO:1 that is at least 12 nucleotides long). The probe will selectively hybridize to nucleic acids encoding related polypeptides (or to complementary sequences thereof). Because the polypeptide encoded by Tango-67 is related to other C-C chemokines, the term "selectively hybridize" is used to refer to an event in which a probe binds to nucleic acids encoding Tango-67 (or to complementary sequences thereof) to a detectably greater extent than to nucleic acids encoding other C-C chemokines (or to complementary sequences thereof). The probe, which can contain at least 12 (for example, 15, 25, 50, 100, or 200 nucleotides) can be produced using any of several standard methods (see, for example, Ausubel et al.,"Current Protocols in Molecular Biology, Vol. I," Green Publishing Associates, Inc., and John Wiley & Sons, Inc., NY, 1989). For example, the probe

can be generated using PCR amplification methods in which oligonucleotide primers are used to amplify a Tango-67-specific nucleic acid sequence (for example, a nucleic acid encoding the chemokine-like domain) that can be used as a probe to screen a nucleic acid library, as described in Example 1 below, and thereby detect nucleic acid molecules (within the library) that hybridize to the probe.

One single-stranded nucleic acid is said to hybridize to another if a duplex forms between them. This occurs when one nucleic acid contains a sequence that is the reverse and complement of the other (this same arrangement gives rise to the natural interaction between the sense and antisense strands of DNA in the genome and underlies the configuration of the "double helix"). Complete complementarity between the hybridizing regions is not required in order for a duplex to form; it is only necessary that the number of paired bases is sufficient to maintain the duplex under the hybridization conditions used.

Typically, hybridization conditions are of low to moderate stringency. These conditions favor specific interactions between completely complementary sequences, but allow some non-specific interaction between less than perfectly matched sequences to occur as well. After hybridization, the nucleic acids can be "washed" under moderate or high conditions of stringency to dissociate duplexes that are bound together by some non-specific interaction (the nucleic acids that form these duplexes are thus not completely complementary).

As is known in the art, the optimal conditions for washing are determined empirically, often by gradually increasing the stringency. The parameters that can be changed to affect stringency include, primarily, temperature and salt concentration. In general, the

lower the salt concentration and the higher the temperature, the higher the stringency. Washing can be initiated at a low temperature (for example, room temperature) using a solution containing a salt concentration that is equivalent to or lower than that of the hybridization solution. Subsequent washing can be carried out using progressively warmer solutions having the same salt concentration. As alternatives, the salt concentration can be lowered and the temperature maintained in the washing step, or the salt concentration can be lowered and the temperature increased. Additional parameters can also be altered. For example, use of a destabilizing agent, such as formamide, alters the stringency conditions.

In reactions where nucleic acids are hybridized, the conditions used to achieve a given level of stringency will vary. There is not one set of conditions, for example, that will allow duplexes to form between all nucleic acids that are 85% identical to one another; hybridization also depends on unique features of each nucleic acid. The length of the sequence, the composition of the sequence (for example, the content of purine-like nucleotides versus the content of pyrimidine-like nucleotides) and the type of nucleic acid (for example, DNA or RNA) affect hybridization. An additional consideration is whether one of the nucleic acids is immobilized (for example, on a filter).

An example of a progression from lower to higher stringency conditions is the following, where the salt content is given as the relative abundance of SSC (a salt solution containing sodium chloride and sodium citrate; 2X SSC is 10-fold more concentrated than 0.2X SSC). Nucleic acids are hybridized at 42°C in 2X SSC/0.1% SDS (sodium dodecylsulfate; a detergent) and then washed in 0.2X SSC/0.1% SDS at room temperature (for conditions of

low stringency); 0.2X SSC/0.1% SDS at 42°C (for conditions of moderate stringency); and 0.1X SSC at 68°C (for conditions of high stringency). Washing can be carried out using only one of the conditions given, or each of the conditions can be used (for example, washing for 10-15 minutes each in the order listed above. Any or all of the washes can be repeated. As mentioned above, optimal conditions will vary and can be determined empirically.

A second set of conditions that are considered "stringent conditions" are those in which hybridization is carried out at 50°C in Church buffer (7% SDS, 0.5% $NaHPO_4$, 1 M EDTA, 1% BSA) and washing is carried out at 50°C in 2X SSC.

Once detected, the nucleic acid molecules can be isolated by any of a number of standard techniques (see, for example, Sambrook et al., "Molecular Cloning, A Laboratory Manual," 2nd Ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989).

The invention also encompasses: (a) expression vectors that contain any of the foregoing Tango-67-related coding sequences and/or their complements (that is, "antisense" sequence); (b) expression vectors that contain any of the foregoing Tango-67-related coding sequences operatively associated with a regulatory element (examples of which are given below) that directs the expression of the coding sequences; (c) expression vectors containing, in addition to sequences encoding a Tango-67 polypeptide, nucleic acid sequences that are unrelated to nucleic acid sequences encoding Tango-67, such as molecules encoding a reporter or marker; and (d) genetically engineered host cells that contain any of the foregoing expression vectors and thereby express the nucleic acid molecules of the invention in the host cell.

Recombinant nucleic acid molecule can contain a sequence encoding a soluble Tango-67 polypeptide, mature Tango-67, Tango-67 having a signal sequence, or the chemokine-like domain of Tango-67. The full length Tango-67 polypeptide, a domain of Tango-67, or a fragment thereof may be fused to additional polypeptides, as described below. Similarly, the nucleic acid molecules of the invention can encode the mature form of Tango-67 or a form that encodes a polypeptide which facilitates secretion. In the latter instance, the polypeptide is typically referred to as a proprotein, which can be converted into an active form by removal of the signal sequence, for example, within the host cell. Proproteins can be converted into the active form of the protein by removal of the inactivating sequence.

The regulatory elements referred to above include, but are not limited to, inducible and non-inducible promoters, enhancers, operators and other elements, which are known to those skilled in the art, and which drive or otherwise regulate gene expression. Such regulatory elements include but are not limited to the cytomegalovirus hCMV immediate early gene, the early or late promoters of SV40 adenovirus, the lac system, the trp system, the TAC system, the TRC system, the major operator and promoter regions of phage A, the control regions of fd coat protein, the promoter for 3-phosphoglycerate kinase, the promoters of acid phosphatase, and the promoters of the yeast α-mating factors.

Similarly, the nucleic acid can form part of a hybrid gene encoding additional polypeptide sequences, for example, sequences that function as a marker or reporter. Examples of marker or reporter genes include β-lactamase, chloramphenicol acetyltransferase (CAT), adenosine deaminase (ADA), aminoglycoside

phosphotransferase (neo^r, G418^r), dihydrofolate reductase (DHFR), hygromycin-B-phosphotransferase (HPH), thymidine kinase (TK), lacZ (encoding β-galactosidase), and xanthine guanine phosphoribosyltransferase (XGPRT). As with many of the standard procedures associated with the practice of the invention, skilled artisans will be aware of additional useful reagents, for example, of additional sequences that can serve the function of a marker or reporter. Generally, the hybrid polypeptide will include a first portion and a second portion; the first portion being a Tango-67 polypeptide and the second portion being, for example, the reporter described above or an immunoglobulin constant region.

The expression systems that may be used for purposes of the invention include, but are not limited to, microorganisms such as bacteria (for example, *E. coli* and *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA, or cosmid DNA expression vectors containing the nucleic acid molecules of the invention; yeast (for example, *Saccharomyces* and *Pichia*) transformed with recombinant yeast expression vectors containing the nucleic acid molecules of the invention (preferably containing the nucleic acid sequence encoding Tango-67 (contained within SEQ ID NO:1)); insect cell systems infected with recombinant virus expression vectors (for example, baculovirus) containing the nucleic acid molecules of the invention; plant cell systems infected with recombinant virus expression vectors (for example, cauliflower mosaic virus (CaMV) and tobacco mosaic virus (TMV)) or transformed with recombinant plasmid expression vectors (for example, Ti plasmid) containing Tango-67 nucleotide sequences; or mammalian cell systems (for example, COS, CHO, BHK, 293, VERO, HeLa, MDCK, WI38, and NIH 3T3 cells) harboring recombinant expression constructs containing promoters

derived from the genome of mammalian cells (for example, the metallothionein promoter) or from mammalian viruses (for example, the adenovirus late promoter and the vaccinia virus 7.5K promoter).

In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the gene product being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions containing Tango-67 polypeptides or for raising antibodies to those polypeptides, vectors that are capable of directing the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited to, the *E. coli* expression vector pUR278 (Ruther et al., *EMBO J.* 2:1791, 1983), in which the coding sequence of the insert may be ligated individually into the vector in frame with the lacZ coding region so that a fusion protein is produced; pIN vectors (Inouye and Inouye, *Nucleic Acids Res.* 13:3101-3109, 1985; Van Heeke and Schuster, *J. Biol. Chem.* 264:5503-5509, 1989); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

In an insect system, *Autographa californica* nuclear polyhidrosis virus (AcNPV) can be used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The coding sequence of the insert may be cloned individually into non-essential

regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter). Successful insertion of the coding sequence will result in inactivation of the polyhedrin gene and production of non-occluded recombinant virus (i.e., virus lacking the proteinaceous coat coded for by the polyhedrin gene). These recombinant viruses are then used to infect *Spodoptera frugiperda* cells in which the inserted gene is expressed. (for example, see Smith et al., *J. Virol.* 46:584, 1983; Smith, U.S. Patent No. 4,215,051).

In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the nucleic acid molecule of the invention may be ligated to an adenovirus transcription/translation control complex, for example, the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (for example, region E1 or E3) will result in a recombinant virus that is viable and capable of expressing a Tango-67 gene product in infected hosts (for example, see Logan and Shenk, *Proc. Natl. Acad. Sci. USA* 81:3655-3659, 1984). Specific initiation signals may also be required for efficient translation of inserted nucleic acid molecules. These signals include the ATG initiation codon and adjacent sequences. In cases where an entire gene or cDNA, including its own initiation codon and adjacent sequences, is inserted into the appropriate expression vector, no additional translational control signals may be needed. However, in cases where only a portion of the coding sequence is inserted, exogenous translational control signals, including, perhaps, the ATG initiation codon, must be

provided. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see Bittner et al., *Methods in Enzymol.* 153:516-544, 1987).

In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (for example, glycosylation) and processing (for example, cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. The mammalian cell types listed above are among those that could serve as suitable host cells.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express the Tango-67 sequences described above may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (for example, promoter, enhancer sequences, transcription

terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method can advantageously be used to engineer cell lines which express Tango-67. Such engineered cell lines may be particularly useful in screening and evaluation of compounds that affect the endogenous activity of the gene product.

A number of selection systems can be used. For example, the herpes simplex virus thymidine kinase (Wigler, et al., *Cell* 11:223, 1977), hypoxanthine-guanine phosphoribosyltransferase (Szybalska and Szybalski, *Proc. Natl. Acad. Sci. USA* 48:2026, 1962), and adenine phosphoribosyltransferase (Lowy, et al., *Cell* 22:817, 1980) genes can be employed in tk^-, $hgprt^-$ or $aprt^-$ cells, respectively. Also, anti-metabolite resistance can be used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate (Wigler et al., *Proc. Natl. Acad. Sci. USA* 77:3567, 1980; O'Hare et al., *Proc. Natl. Acad. Sci. USA* 78:1527, 1981); gpt, which confers resistance to mycophenolic acid (Mulligan and Berg, *Proc. Natl. Acad. Sci. USA* 78:2072, 1981); neo, which confers resistance to the aminoglycoside G-418 (Colberre-Garapin et al., *J. Mol. Biol.* 150:1, 1981); and hygro, which confers resistance to hygromycin (Santerre et al., *Gene* 30:147, 1984).

Alternatively, any fusion protein may be readily purified by utilizing an antibody specific for the fusion protein being expressed. For example, a system described

by Janknecht et al. allows for the ready purification of non-denatured fusion proteins expressed in human cell lines (*Proc. Natl. Acad. Sci. USA* 88: 8972-8976, 1991). In this system, the gene of interest is subcloned into a vaccinia recombination plasmid such that the gene's open reading frame is translationally fused to an amino-terminal tag consisting of six histidine residues. Extracts from cells infected with recombinant vaccinia virus are loaded onto Ni^{2+}·nitriloacetic acid-agarose columns and histidine-tagged proteins are selectively eluted with imidazole-containing buffers.

Tango-67 nucleic acid molecules are useful for diagnosis of disorders associated with aberrant expression of Tango-67. Tango-67 nucleic acid molecules are useful in genetic mapping.

Tango-67 Polypeptides

The Tango-67 polypeptides described herein are those encoded by any of the nucleic acid molecules described above and include Tango-67 fragments, mutants, truncated forms, and fusion proteins. These polypeptides can be prepared for a variety of uses, including but not limited to the generation of antibodies, as reagents in diagnostic assays, for the identification of other cellular gene products or compounds that can modulate the activity or expression of Tango-67, and as pharmaceutical reagents useful for the treatment of disorders associated with aberrant expression or activity of Tango-67.

Preferred polypeptides are substantially pure Tango-67 polypeptides, including those that correspond to the polypeptide with an intact signal sequence, the secreted form of the polypeptide of the human Tango-67 polypeptide. Especially preferred are polypeptides that are soluble under normal physiological conditions.

The invention also encompasses polypeptides that are functionally equivalent to Tango-67. These polypeptides are equivalent to Tango-67 in that they are capable of carrying out one or more of the functions of Tango-67 in a biological system. Preferred Tango-67 polypeptides have 20%, 40%, 50%, 75%, 80%, or even 90% of the activity of the full-length, mature human form of Tango-67. Such comparisons are generally based on an assay of biological activity in which equal concentrations of the polypeptides are used and compared. The comparison can also be based on the amount of the polypeptide required to reach 50% of the maximal stimulation obtainable.

Functionally equivalent proteins can be those, for example, that contain additional or substituted amino acid residues. Substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues involved. Amino acids that are typically considered to provide a conservative substitution for one another are specified in the summary of the invention.

Polypeptides that are functionally equivalent to Tango-67 (SEQ ID NO:2) can be made using random mutagenesis techniques well known to those skilled in the art (and the resulting mutant Tango-67 proteins can be tested for activity). It is more likely, however, that such polypeptides will be generated by site-directed mutagenesis (again using techniques well known to those skilled in the art). These polypeptides may have an increased function, *i.e.*, a greater ability to inhibit cellular proliferation, or to evoke an inflammatory response. Such polypeptides can be used to protect progenitor cells from the effects of chemotherapy and/or radiation therapy.

To design functionally equivalent polypeptides, it is useful to distinguish between conserved positions and variable positions. This can be done by aligning the sequence of Tango-67 cDNAs that were obtained from various organisms. Skilled artisans will recognize that conserved amino acid residues are more likely to be necessary for preservation of function. Thus, it is preferable that conserved residues are not altered.

Mutations within the Tango-67 coding sequence can be made to generate Tango-67s that are better suited for expression in a selected host cell. For example, N-linked glycosylation sites can be altered or eliminated to achieve, for example, expression of a homogeneous product that is more easily recovered and purified from yeast hosts which are known to hyperglycosylate N-linked sites. To this end, a variety of amino acid substitutions at one or both of the first or third amino acid positions of any one or more of the glycosylation recognition sequences which occur (in N-X-S or N-X--), and/or an amino acid deletion at the second position of any one or more of such recognition sequences, will prevent glycosylation at the modified tripeptide sequence (see, for example, Miyajima et al., *EMBO J.* 5:1193, 1986).

The polypeptides of the invention can be expressed fused to another polypeptide, for example, a marker polypeptide or fusion partner. For example, the polypeptide can be fused to a hexa-histidine tag to facilitate purification of bacterially expressed protein or a hemagglutinin tag to facilitate purification of protein expressed in eukaryotic cells.

The polypeptides of the invention can be chemically synthesized (for example, see Creighton, "Proteins: Structures and Molecular Principles," W.H. Freeman & Co., NY, 1983), or, perhaps more

advantageously, produced by recombinant DNA technology as described herein. For additional guidance, skilled artisans may consult Ausubel et al. (*supra*), Sambrook et al. ("Molecular Cloning, A Laboratory Manual," Cold Spring Harbor Press, Cold Spring Harbor, NY, 1989), and, particularly for examples of chemical synthesis Gait, M.J. Ed. ("Oligonucleotide Synthesis," IRL Press, Oxford, 1984).

The invention also features polypeptides that interact with Tango-67 (and the genes that encode them) and thereby alter the function of Tango-67. Interacting polypeptides can be identified using methods known to those skilled in the art. One suitable method is the "two-hybrid system," which detects protein interactions *in vivo* (Chien et al., *Proc. Natl. Acad. Sci. USA*, 88:9578, 1991). A kit for practicing this method is available from Clontech (Palo Alto, CA).

Tango-67 polypeptides are useful for growth promotion. Accordingly they have applications in wound healing, tissue repair, implant fixation, and stimulation of bone growth.

Transgenic animals

Tango-67 polypeptides can also be expressed in transgenic animals. These animals represent a model system for the study of disorders that are caused by or exacerbated by overexpression or underexpression of Tango-67, and for the development of therapeutic agents that modulate the expression or activity of Tango-67.

Transgenic animals can be farm animals (pigs, goats, sheep, cows, horses, rabbits, and the like) rodents (such as rats, guinea pigs, and mice), non-human primates (for example, baboons, monkeys, and chimpanzees), and domestic animals (for example, dogs and cats). Transgenic mice are especially preferred.

Any technique known in the art can be used to introduce a Tango-67 transgene into animals to produce the founder lines of transgenic animals. Such techniques include, but are not limited to, pronuclear microinjection (U.S. Pat. No. 4,873,191); retrovirus mediated gene transfer into germ lines (Van der Putten et al., *Proc. Natl. Acad. Sci., USA* 82:6148, 1985); gene targeting into embryonic stem cells (Thompson et al., *Cell* 56:313, 1989); and electroporation of embryos (Lo, *Mol. Cell. Biol.* 3:1803, 1983).

The present invention provides for transgenic animals that carry the Tango-67 transgene in all their cells, as well as animals that carry the transgene in some, but not all of their cells. That is, the invention provides for mosaic animals. The transgene can be integrated as a single transgene or in concatamers, e.g., head-to-head tandems or head-to-tail tandems. The transgene can also be selectively introduced into and activated in a particular cell type (Lasko et al., *Proc. Natl. Acad. Sci. USA* 89:6232, 1992). The regulatory sequences required for such a cell-type specific activation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art.

When it is desired that the Tango-67 transgene be integrated into the chromosomal site of the endogenous Tango-67 gene, gene targeting is preferred. Briefly, when such a technique is to be used, vectors containing some nucleotide sequences homologous to an endogenous Tango-67 gene are designed for the purpose of integrating, via homologous recombination with chromosomal sequences, into and disrupting the function of the nucleotide sequence of the endogenous gene. The transgene also can be selectively introduced into a particular cell type, thus inactivating the endogenous

Tango-67 gene in only that cell type (Gu et al., *Science* 265:103, 1984). The regulatory sequences required for such a cell-type specific inactivation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art. These techniques are useful for preparing "knock outs" having no functional Tango-67 gene.

Once transgenic animals have been generated, the expression of the recombinant Tango-67 gene can be assayed utilizing standard techniques. Initial screening may be accomplished by Southern blot analysis or PCR techniques to determine whether integration of the transgene has taken place. The level of mRNA expression of the transgene in the tissues of the transgenic animals may also be assessed using techniques which include, but are not limited to, Northern blot analysis of tissue samples obtained from the animal, *in situ* hybridization analysis, and RT-PCR. Samples of Tango-67 gene-expressing tissue can also be evaluated immunocytochemically using antibodies specific for the Tango-67 transgene product.

For a review of techniques that can be used to generate and assess transgenic animals, skilled artisans can consult Gordon (*Intl. Rev. Cytol.* 115:171-229, 1989), and may obtain additional guidance from, for example: Hogan et al. "Manipulating the Mouse Embryo" (Cold Spring Harbor Press, Cold Spring Harbor, NY, 1986; Krimpenfort et al., *Bio/Technology* 9:86, 1991; Palmiter et al., *Cell* 41:343, 1985; Kraemer et al., "Genetic Manipulation of the Early Mammalian Embryo," Cold Spring Harbor Press, Cold Spring Harbor, NY, 1985; Hammer et al., *Nature* 315:680, 1985; Purcel et al., *Science*, 244:1281, 1986; Wagner et al., U.S. Patent No. 5,175,385; and Krimpenfort et al., U.S. Patent No. 5,175,384 (the latter two publications are hereby incorporated by reference).

Anti-Tango-67 Antibodies

Human Tango-67 polypeptides (or immunogenic fragments or analogs) can be used to raise antibodies useful in the invention; such polypeptides can be produced by recombinant techniques or synthesized (see, for example, "Solid Phase Peptide Synthesis," *supra*; Ausubel et al., *supra*). In general, the peptides can be coupled to a carrier protein, such as KLH, as described in Ausubel et al., *supra*, mixed with an adjuvant, and injected into a host mammal. Antibodies can be purified by peptide antigen affinity chromatography.

In particular, various host animals can be immunized by injection with a Tango-67 protein or polypeptide. Host animals include rabbits, mice, guinea pigs, and rats. Various adjuvants that can be used to increase the immunological response depend on the host species and include Freund's adjuvant (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, and dinitrophenol. Potentially useful human adjuvants include BCG (bacille Calmette-Guerin) and *Corynebacterium parvum*. Polyclonal antibodies are heterogeneous populations of antibody molecules that are contained in the sera of the immunized animals.

Antibodies within the invention therefore include polyclonal antibodies and, in addition, monoclonal antibodies, humanized or chimeric antibodies, single chain antibodies, Fab fragments, $F(ab')_2$ fragments, and molecules produced using a Fab expression library.

Monoclonal antibodies, which are homogeneous populations of antibodies to a particular antigen, can be prepared using the Tango-67 proteins described above and standard hybridoma technology (see, for example, Kohler et al., *Nature* 256:495, 1975; Kohler et al., *Eur. J.*

Immunol. 6:511, 1976; Kohler et al., *Eur. J. Immunol.* 6:292, 1976; Hammerling et al., "Monoclonal Antibodies and T Cell Hybridomas," Elsevier, NY, 1981; Ausubel et al., *supra*).

In particular, monoclonal antibodies can be obtained by any technique that provides for the production of antibody molecules by continuous cell lines in culture such as described in Kohler et al., *Nature* 256:495, 1975, and U.S. Patent No. 4,376,110; the human B-cell hybridoma technique (Kosbor et al., *Immunology Today* 4:72, 1983; Cole et al., *Proc. Natl. Acad. Sci. USA* 80:2026, 1983), and the EBV-hybridoma technique (Cole et al., "Monoclonal Antibodies and Cancer Therapy," Alan R. Liss, Inc., pp. 77-96, 1983). Such antibodies can be of any immunoglobulin class including IgG, IgM, IgE, IgA, IgD and any subclass thereof. The hybridoma producing the mAb of this invention may be cultivated *in vitro* or *in vivo*. The ability to produce high titers of mAbs *in vivo* makes this the presently preferred method of production.

Once produced, polyclonal or monoclonal antibodies are tested for specific Tango-67 recognition by Western blot or immunoprecipitation analysis by standard methods, e.g., as described in Ausubel et al., *supra*. Antibodies that specifically recognize and bind to Tango-67 are useful in the invention. For example, such antibodies can be used in an immunoassay to monitor the level of Tango-67 produced by a mammal (for example, to determine the amount or subcellular location of Tango-67).

Preferably, antibodies of the invention are produced using fragments of the Tango-67 protein which lie outside highly conserved regions and appear likely to be antigenic, by criteria such as high frequency of charged residues. In one specific example, such fragments are generated by standard techniques of PCR,

and are then cloned into the pGEX expression vector (Ausubel et al., *supra*). Fusion proteins are expressed in *E. coli* and purified using a glutathione agarose affinity matrix as described in Ausubel, et al., *supra*.

In some cases it may be desirable to minimize the potential problems of low affinity or specificity of antisera. In such circumstances, two or three fusions can be generated for each protein, and each fusion can be injected into at least two rabbits. Antisera can be raised by injections in a series, preferably including at least three booster injections.

Antisera is also checked for its ability to immunoprecipitate recombinant Tango-67 proteins or control proteins, such as glucocorticoid receptor, CAT, or luciferase.

The antibodies can be used, for example, in the detection of the Tango-67 in a biological sample as part of a diagnostic assay. Antibodies also can be used in a screening assay to measure the effect of a candidate compound on expression or localization of Tango-67. Additionally, such antibodies can be used in conjunction with the gene therapy techniques described to, for example, evaluate the normal and/or engineered Tango-67-expressing cells prior to their introduction into the patient. Such antibodies additionally can be used in a method for inhibiting abnormal Tango-67 activity.

In addition, techniques developed for the production of "chimeric antibodies" (Morrison et al., *Proc. Natl. Acad. Sci. USA*, 81:6851, 1984; Neuberger et al., *Nature*, 312:604, 1984; Takeda et al., *Nature*, 314:452, 1984) by splicing the genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. A chimeric antibody is a molecule in which different portions are

derived from different animal species, such as those having a variable region derived from a murine mAb and a human immunoglobulin constant region.

Alternatively, techniques described for the production of single chain antibodies (U.S. Patent Nos. 4,946,778, 4,946,778, and 4,704,692) can be adapted to produce single chain antibodies against a Tango-67 protein or polypeptide. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide.

Antibody fragments that recognize and bind to specific epitopes can be generated by known techniques. For example, such fragments include but are not limited to $F(ab')_2$ fragments that can be produced by pepsin digestion of the antibody molecule, and Fab fragments that can be generated by reducing the disulfide bridges of $F(ab')_2$ fragments. Alternatively, Fab expression libraries can be constructed (Huse et al., *Science*, 246:1275, 1989) to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity.

Antibodies to Tango-67 can, in turn, be used to generate anti-idiotype antibodies that resemble a portion of Tango-67 using techniques well known to those skilled in the art (see, e.g., Greenspan et al., *FASEB J.* 7:437, 1993; Nissinoff, *J. Immunol.* 147:2429, 1991). For example, antibodies that bind to Tango-67 and competitively inhibit the binding of a ligand of Tango-67 can be used to generate anti-idiotypes that resemble a ligand binding domain of Tango-67 and, therefore, bind and neutralize a ligand of Tango-67. Such neutralizing anti-idiotypic antibodies or Fab fragments of such anti-idiotypic antibodies can be used in therapeutic regimens.

Antibodies can be humanized by methods known in the art. For example, monoclonal antibodies with a

desired binding specificity can be commercially humanized (Scotgene, Scotland; Oxford Molecular, Palo Alto, CA). Fully human antibodies, such as those expressed in transgenic animals are also features of the invention (Green et al., Nature Genetics 7:13-21, 1994; see also U.S. Patents 5,545,806 and 5,569,825, both of which are hereby incorporated by reference).

The methods described herein in which anti-Tango-67 antibodies are employed may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one specific Tango-67 nucleotide sequence or antibody reagent described herein, which may be conveniently used, for example, in clinical settings, to diagnose patients exhibiting symptoms of the disorders described below.

Antisense Nucleic Acids

Treatment regimes based on an "antisense" approach involve the design of oligonucleotides (either DNA or RNA) that are complementary to Tango-67 mRNA. These oligonucleotides bind to the complementary Tango-67 mRNA transcripts and prevent translation. Absolute complementarity, although preferred, is not required. A sequence "complementary" to a portion of an RNA, as referred to herein, means a sequence having sufficient complementarily to be able to hybridize with the RNA, forming a stable duplex; in the case of double-stranded antisense nucleic acids, a single strand of the duplex DNA may be tested, or triplex formation may be assayed. The ability to hybridize will depend on both the degree of complementarily and the length of the antisense nucleic acid. Generally, the longer the hybridizing nucleic acid, the more base mismatches with an RNA it may contain and still form a stable duplex (or triplex, as the case may be). One skilled in the art can ascertain a

tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

Oligonucleotides that are complementary to the 5' end of the message, e.g., the 5' untranslated sequence up to and including the AUG initiation codon, should work most efficiently at inhibiting translation. However, sequences complementary to the 3' untranslated sequences of mRNAs recently have been shown to be effective at inhibiting translation of mRNAs as well (Wagner, *Nature* 372:333, 1984). Thus, oligonucleotides complementary to either the 5' or 3' non-translated, non-coding regions of the Tango-67 gene, e.g., the human gene shown in the Figure could be used in an antisense approach to inhibit translation of endogenous Tango-67 mRNA. Oligonucleotides complementary to the 5' untranslated region of the mRNA should include the complement of the AUG start codon.

Antisense oligonucleotides complementary to mRNA coding regions are less efficient inhibitors of translation but could be used in accordance with the invention. Whether designed to hybridize to the 5', 3', or coding region of Tango-67 mRNA, antisense nucleic acids should be at least six nucleotides in length, and are preferably oligonucleotides ranging from 6 to about 50 nucleotides in length. In specific aspects the oligonucleotide is at least 10 nucleotides, at least 17 nucleotides, at least 25 nucleotides, or at least 50 nucleotides.

Regardless of the choice of target sequence, it is preferred that *in vitro* studies are first performed to quantitate the ability of the antisense oligonucleotide to inhibit gene expression. It is preferred that these studies utilize controls that distinguish between antisense gene inhibition and nonspecific biological

effects of oligonucleotides. It is also preferred that these studies compare levels of the target RNA or protein with that of an internal control RNA or protein. Additionally, it is envisioned that results obtained using the antisense oligonucleotide are compared with those obtained using a control oligonucleotide. It is preferred that the control oligonucleotide is of approximately the same length as the test oligonucleotide and that the nucleotide sequence of the oligonucleotide differs from the antisense sequence no more than is necessary to prevent specific hybridization to the target sequence.

The oligonucleotides can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone, for example, to improve stability of the molecule, hybridization, etc. The oligonucleotide may include other appended groups such as peptides (e.g., for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (as described, e.g., in Letsinger et al., *Proc. Natl. Acad. Sci. USA* 86:6553, 1989; Lemaitre et al., *Proc. Natl. Acad. Sci. USA* 84:648, 1987; PCT Publication No. WO 88/09810) or the blood-brain barrier (see, for example, PCT Publication No. WO 89/10134), or hybridization-triggered cleavage agents (see, for example, Krol et al., *BioTechniques* 6:958, 1988), or intercalating agents (see, for example, Zon, *Pharm. Res.* 5:539, 1988). To this end, the oligonucleotide can be conjugated to another molecule, e.g., a peptide, hybridization triggered cross-linking agent, transport agent, or hybridization-triggered cleavage agent.

The antisense oligonucleotide may comprise at least one modified base moiety which is selected from the

group including, but not limited to, 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethyl-aminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-theouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 2-(3-amino-3-N-2-carboxypropl) uracil, (acp3)w, and 2,6-diaminopurine.

The antisense oligonucleotide may also comprise at least one modified sugar moiety selected from the group including, but not limited to, arabinose, 2-fluoroarabinose, xylulose, and hexose.

In yet another embodiment, the antisense oligonucleotide comprises at least one modified phosphate backbone selected from the group consisting of a phosphorothioate, a phosphorodithioate, a phosphoramidothioate, a phosphoramidate, a phosphordiamidate, a methylphosphonate, an alkyl phosphotriester, and a formacetal, or an analog of any of these backbones.

In yet another embodiment, the antisense oligonucleotide is an α-anomeric oligonucleotide. An α-anomeric oligonucleotide forms specific double-stranded

hybrids with complementary RNA in which, contrary to the usual β-units, the strands run parallel to each other (Gautier et al., *Nucl. Acids. Res.* 15:6625, 1987). The oligonucleotide is a 2'-0-methylribonucleotide (Inoue et al., *Nucl. Acids Res.* 15:6131, 1987), or a chimeric RNA-DNA analog (Inoue et al., *FEBS Lett.* 215:327, 1987).

Antisense oligonucleotides of the invention can be synthesized by standard methods known in the art, e.g., by use of an automated DNA synthesizer (such as are commercially available from Biosearch, Applied Biosystems, etc.). As examples, phosphorothioate oligonucleotides can be synthesized by the method of Stein et al. (*Nucl. Acids Res.* 16:3209, 1988), and methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer supports (Sarin et al., *Proc. Natl. Acad. Sci. USA* 85:7448, 1988).

While antisense nucleotides complementary to the Tango-67 coding region sequence could be used, those complementary to the transcribed untranslated region are most preferred.

The antisense molecules should be delivered to cells that express Tango-67 *in vivo*, e.g., cells of the heart, skeletal muscle, thymus, spleen, and small intestine. A number of methods have been developed for delivering antisense DNA or RNA to cells; e.g., antisense molecules can be injected directly into the tissue site, or modified antisense molecules, designed to target the desired cells (e.g., antisense linked to peptides or antibodies that specifically bind receptors or antigens expressed on the target cell surface) can be administered systemically.

However, it is often difficult to achieve intracellular concentrations of the antisense molecule sufficient to suppress translation of endogenous mRNAs. Therefore, a preferred approach uses a recombinant DNA

construct in which the antisense oligonucleotide is placed under the control of a strong *pol* III or *pol* II promoter. The use of such a construct to transfect target cells in the patient will result in the transcription of sufficient amounts of single stranded RNAs that will form complementary base pairs with the endogenous Tango-67 transcripts and thereby prevent translation of the Tango-67 mRNA. For example, a vector can be introduced *in vivo* such that it is taken up by a cell and directs the transcription of an antisense RNA. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA.

Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors can be plasmid, viral, or others known in the art, used for replication and expression in mammalian cells. Expression of the sequence encoding the antisense RNA can be by any promoter known in the art to act in mammalian, preferably human cells. Such promoters can be inducible or constitutive. Such promoters include, but are not limited to: the SV40 early promoter region (Bernoist et al., *Nature* 290:304, 1981); the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto et al., *Cell* 22:787-797, 1988); the herpes thymidine kinase promoter (Wagner et al., *Proc. Natl. Acad. Sci. USA* 78:1441, 1981); or the regulatory sequences of the metallothionein gene (Brinster et al., *Nature* 296:39, 1988).

Ribozymes

Ribozyme molecules designed to catalytically cleave Tango-67 mRNA transcripts also can be used to prevent translation of Tango-67 mRNA and expression of Tango-67 (see, e.g., PCT Publication WO 90/11364; Saraver et al., *Science* 247:1222, 1990). While various ribozymes

that cleave mRNA at site-specific recognition sequences can be used to destroy Tango-67 mRNAs, the use of hammerhead ribozymes is preferred. Hammerhead ribozymes cleave mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA. The sole requirement is that the target mRNA have the following sequence of two bases: 5'-UG-3'. The construction and production of hammerhead ribozymes is well known in the art (Haseloff et al., *Nature* 334:585, 1988). There are numerous examples of potential hammerhead ribozyme cleavage sites within the nucleotide sequence of human Tango-67 cDNA. Preferably, the ribozyme is engineered so that the cleavage recognition site is located near the 5' end of the Tango-67 mRNA, i.e., to increase efficiency and minimize the intracellular accumulation of non-functional mRNA transcripts.

The ribozymes of the present invention also include RNA endoribonucleases (hereinafter "Cech-type ribozymes"), such as the one that occurs naturally in *Tetrahymena Thermophila* (known as the IVS or L-19 IVS RNA), and which has been extensively described by Cech and his collaborators (Zaug et al., *Science* 224:574, 1984; Zaug et al., *Science*, 231:470, 1986; Zug et al., *Nature* 324:429, 1986; PCT Application No. WO 88/04300; and Been et al., *Cell* 47:207, 1986). The Cech-type ribozymes have an eight base-pair sequence that hybridizes to a target RNA sequence, whereafter cleavage of the target RNA takes place. The invention encompasses those Cech-type ribozymes that target eight base-pair active site sequences present in Tango-67.

As in the antisense approach, the ribozymes can be composed of modified oligonucleotides (e.g., for improved stability, targeting, etc.), and should be delivered to cells which express the Tango-67 *in vivo*, e.g., heart,

skeletal muscle, thymus, spleen, and small intestine. A preferred method of delivery involves using a DNA construct "encoding" the ribozyme under the control of a strong constitutive *pol* III or *pol* II promoter, so that transfected cells will produce sufficient quantities of the ribozyme to destroy endogenous Tango-67 messages and inhibit translation. Because ribozymes, unlike antisense molecules, are catalytic, a lower intracellular concentration is required for efficiency.

Other Methods for Reducing Tango-67 Expression

Endogenous Tango-67 gene expression can also be reduced by inactivating or "knocking out" the Tango-67 gene or its promoter using targeted homologous recombination (see, e.g., U.S. Patent No. 5,464,764). For example, a mutant, non-functional Tango-67 (or a completely unrelated DNA sequence) flanked by DNA homologous to the endogenous Tango-67 gene (either the coding regions or regulatory regions of the Tango-67 gene) can be used, with or without a selectable marker and/or a negative selectable marker, to transfect cells that express Tango-67 *in vivo*. Insertion of the DNA construct, via targeted homologous recombination, results in inactivation of the Tango-67 gene. Such approaches are particularly suited for use in the agricultural field where modifications to ES (embryonic stem) cells can be used to generate animal offspring with an inactive Tango-67. However, this approach can be adapted for use in humans, provided the recombinant DNA constructs are directly administered or targeted to the required site *in vivo* using appropriate viral vectors.

Alternatively, endogenous Tango-67 gene expression can be reduced by targeting deoxyribonucleotide sequences complementary to the regulatory region of the Tango-67 gene (*i.e.*, the Tango-67 promoter and/or enhancers) to

form triple helical structures that prevent transcription of the Tango-67 gene in target cells in the body (Helene *Anticancer Drug Res.* 6:569, 1981; Helene et al., *Ann. N.Y. Acad. Sci.* 660:27, 1992; and Maher, *Bioassays* 14:807, 1992).

Of course, in some circumstances, including certain phases of many of the above-described conditions, it may be desirable to enhance Tango-67 function, e.g., to recruit immune cells that will resolve the primary infection or mediate an anti-tumor response.

Detecting Proteins Associated with Tango-67

The invention also features polypeptides which interact with Tango-67. Any method suitable for detecting protein-protein interactions may be employed for identifying transmembrane proteins, intracellular, or extracellular proteins that interact with Tango-67. Among the traditional methods which may be employed are co-immunoprecipitation, crosslinking and co-purification through gradients or chromatographic columns of cell lysates or proteins obtained from cell lysates and the use of Tango-67 to identify proteins in the lysate that interact with Tango-67. For these assays, the Tango-67 polypetide can be a full length Tango-67, a soluble extracellular domain of Tango-67, or some other suitable Tango-67 polypeptide. Once isolated, such an interacting protein can be identified and cloned and then used, in conjunction with standard techniques, to identify proteins with which it interacts. For example, at least a portion of the amino acid sequence of a protein which interacts with the Tango-67 can be ascertained using techniques well known to those of skill in the art, such as via the Edman degradation technique. The amino acid sequence obtained may be used as a guide for the generation of oligonucleotide mixtures that can be used

to screen for gene sequences encoding the interacting protein. Screening may be accomplished, for example, by standard hybridization or PCR techniques. Techniques for the generation of oligonucleotide mixtures and the screening are well-known. (Ausubel, *supra;* and "PCR Protocols: A Guide to Methods and Applications," Innis et al., eds. Academic Press, Inc., NY, 1990).

Additionally, methods may be employed which result directly in the identification of genes which encode proteins which interact with Tango-67. These methods include, for example, screening expression libraries, in a manner similar to the well known technique of antibody probing of λgt11 libraries, using labeled Tango-67 polypeptide or a Tango-67 fusion protein, e.g., a Tango-67 polypeptide or domain fused to a marker such as an enzyme, fluorescent dye, a luminescent protein, or to an IgFc domain.

There are also methods which are capable of detecting protein interaction. A method which detects protein interactions *in vivo* is the two-hybrid system (Chien et al., *Proc. Natl. Acad. Sci. USA*, 88:9578, 1991). A kit for practicing this method is available from Clontech (Palo Alto, CA).

Briefly, utilizing such a system, plasmids are constructed that encode two hybrid proteins: one plasmid includes a nucleotide sequence encoding the DNA-binding domain of a transcription activator protein fused to a nucleotide sequence encoding Tango-67, a Tango-67 polypeptide, or a Tango-67 fusion protein, and the other plasmid includes a nucleotide sequence encoding the transcription activator protein's activation domain fused to a cDNA encoding an unknown protein which has been recombined into this plasmid as part of a cDNA library. The DNA-binding domain fusion plasmid and the cDNA library are transformed into a strain of the yeast

Saccharomyces cerevisiae that contains a reporter gene (e.g., HBS or LacZ) whose regulatory region contains the transcription activator's binding site. Either hybrid protein alone cannot activate transcription of the reporter gene: the DNA-binding domain hybrid cannot because it does not provide activation function and the activation domain hybrid cannot because it cannot localize to the activator's binding sites. Interaction of the two hybrid proteins reconstitutes the functional activator protein and results in expression of the reporter gene, which is detected by an assay for the reporter gene product.

The two-hybrid system or related methodology may be used to screen activation domain libraries for proteins that interact with the "bait" gene product. By way of example, and not by way of limitation, Tango-67 may be used as the bait gene product. Total genomic or cDNA sequences are fused to the DNA encoding an activation domain. This library and a plasmid encoding a hybrid of bait Tango-67 gene product fused to the DNA-binding domain are cotransformed into a yeast reporter strain, and the resulting transformants are screened for those that express the reporter gene. For example, a bait Tango-67 gene sequence, such as Tango-67 or a domain of Tango-67 can be cloned into a vector such that it is translationally fused to the DNA encoding the DNA-binding domain of the GAL4 protein. These colonies are purified and the library plasmids responsible for reporter gene expression are isolated. DNA sequencing is then used to identify the proteins encoded by the library plasmids.

A cDNA library of the cell line from which proteins that interact with bait Tango-67 gene product are to be detected can be made using methods routinely practiced in the art. According to the particular system described herein, for example, the cDNA fragments can be

inserted into a vector such that they are translationally fused to the transcriptional activation domain of GAL4. This library can be co-transformed along with the bait Tango-67 gene-GAL4 fusion plasmid into a yeast strain which contains a lacZ gene driven by a promoter which contains GAL4 activation sequence. A cDNA encoded protein, fused to GAL4 transcriptional activation domain, that interacts with bait Tango-67 gene product will reconstitute an active GAL4 protein and thereby drive expression of the HIS3 gene. Colonies which express HIS3 can then be purified from these strains, and used to produce and isolate the bait Tango-67 gene-interacting protein using techniques routinely practiced in the art.

Identification of a Tango-67 Receptor

A Tango-67 receptor can be identified as follows. First cells or tissues which bind Tango-67 are identified. An expression library is prepared using mRNA isolated from Tango-67 binding cells. The expression library is used to transfect; eulcaryatic cells, e.g., CHO cells. Detectably labelled Tango-67 and, clones which bind Tango-67 are isolated and purified. The expression plasmid is then isolated from the Tango-67-binding clones. These expression plasmids will encode putaslive Tango-67 receptors.

Cells or tissues bearing a Tango-67 receptor can be identified by exposing detectably labelled Tango-67 to various cells lines and tissues. Alternatively a microphysiometer can be used to determine whether a selected cells responds to the presence of a cell receptor ligand (McConnel et al., *Science* 257:1906, 1992).

Compounds which bind Tango-67 can be identified using any standard binding assay. For example, candidate compounds can be bound to a solid support. Tango-67 is then exposed to the immobilized compound and binding is measured (European Patent Application 84/03564).

Effective Dose

Toxicity and therapeutic efficacy of the polypeptides of the invention and the compounds that modulate their expression or activity can be determined by standard pharmaceutical procedures, using either cells in culture or experimental animals to determine the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD_{50}/ED_{50}. Polypeptides or other compounds that exhibit large therapeutic indices are preferred. While compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the

IC_{50} (that is, the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

Formulations and Use

Pharmaceutical compositions for use in accordance with the present invention may be formulated in conventional manner using one or more physiologically acceptable carriers or excipients.

Thus, the compounds and their physiologically acceptable salts and solvates may be formulated for administration by inhalation or insufflation (either through the mouth or the nose) or oral, buccal, parenteral or rectal administration.

For oral administration, the pharmaceutical compositions may take the form of, for example, tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (for example, pregelatinised maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (for example, lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (for example, magnesium stearate, talc or silica); disintegrants (for example, potato starch or sodium starch glycolate); or wetting agents (for example, sodium lauryl sulphate). The tablets may be coated by methods well known in the art. Liquid preparations for oral administration may take the form of, for example, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable

additives such as suspending agents (for example, sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (for example, lecithin or acacia); non-aqueous vehicles (for example, almond oil, oily esters, ethyl alcohol or fractionated vegetable oils); and preservatives (for example, methyl or propyl-p-hydroxybenzoates or sorbic acid). The preparations may also contain buffer salts, flavoring, coloring and sweetening agents as appropriate. Preparations for oral administration may be suitably formulated to give controlled release of the active compound.

For buccal administration the compositions may take the form of tablets or lozenges formulated in conventional manner.

For administration by inhalation, the compounds for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, for example, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, for example, gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

The compounds may be formulated for parenteral administration by injection, for example, by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, for example, in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as

suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, for example, sterile pyrogen-free water, before use.

The compounds may also be formulated in rectal compositions such as suppositories or retention enemas, for example, containing conventional suppository bases such as cocoa butter or other glycerides.

In addition to the formulations described previously, the compounds may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the compounds may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

The compositions may, if desired, be presented in a pack or dispenser device which may contain one or more unit dosage forms containing the active ingredient. The pack may for example comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration.

The therapeutic compositions of the invention can also contain a carrier or excipient, many of which are known to skilled artisans. Excipients which can be used include buffers (for example, citrate buffer, phosphate buffer, acetate buffer, and bicarbonate buffer), amino acids, urea, alcohols, ascorbic acid, phospholipids, proteins (for example, serum albumin), EDTA, sodium chloride, liposomes, mannitol, sorbitol, and glycerol. The nucleic acids, polypeptides, antibodies, or modulatory compounds of the invention can be administered by any standard route of administration. For example,

administration can be parenteral, intravenous, subcutaneous, intramuscular, intracranial, intraorbital, opthalmic, intraventricular, intracapsular, intraspinal, intracisternal, intraperitoneal, transmucosal, or oral. The modulatory compound can be formulated in various ways, according to the corresponding route of administration. For example, liquid solutions can be made for ingestion or injection; gels or powders can be made for ingestion, inhalation, or topical application. Methods for making such formulations are well known and can be found in, for example, "Remington's Pharmaceutical Sciences." It is expected that the preferred route of administration will be intravenous.

Examples

Example 1 describes the identification and sequencing of a human Tango-67 gene. Example 2 describes the characterization of Tango-67, particularly the expression pattern.

Example 1: Cloning of the Tango-67 Gene

Human astrocytes (obtained from Clonetics Corporation; San Diego, CA) were expanded in culture with Astrocyte Growth Media (AGN; Clonetics) according to the recommendations of the supplier. When the cells reached ~80-90% confluence, they were stimulated with 200 units/ml Interleukin 1β (Boehringer Mannheim, Indianapolis, IN) and cycloheximide (CHI; 40 micrograms/ml) for 4 hours. Total RNA was isolated using the RNeasy Midi Kit (Qiagen; Chatsworth, CA), and the poly A+ fraction was further purified using Oligotex beads (Qiagen).

Three micrograms of poly A+ RNA were used to synthesize a cDNA library using the Superscript cDNA Synthesis kit (Gibco BRL; Gaithersburg, MD).

Complementary DNA was directionally clones into the expression plasmid pMET7 using the SalI and NotI sites in the polylinker to construct a plasmid library. Transformants were picked and grown up for single-pass sequencing. Additionally, astrocyte cDNA was ligated into the SalI/NotI sites of the ZipLox vector (Gibco BRL) for construction of a lambda phage cDNA library. A partial cDNA clone that encoded a protein with homology to TSG was identified, and additional screening of the phage library led to the isolation of a full length clone for Tango 67. Tango 67 encodes a protein of 223 amino acids that is 36% identical to D. melanogaster TSG, based on comparisons using the GAP program from GCG (Madison, WI).

Example 2: Characterization of Tango-67

The expression pattern of Tango-67 was examined as described below.

Analysis of Tango-67 expression

The expression of Tango 67 was analyzed using Northern blot hybridization. A 410 base pair (bp) DNA fragment was generated using PCR (corresponding to nucleotides 234 to 643 in SEQ ID NO:1) for use as a probe. The DNA was radioactively labeled with ^{32}P-dCTP using the Prime-It kit (Strategen, LaJolla, CA) according to the instructions of the supplier. Filters containing human mRNA (MTNI and MTNII form Clontech, Palo Alto, CA) were probed in ExpressHyb hybridization solution (Clontech) and washed at high stringency according to using a slight variation of the manufacturer's recommendations. The high stringency wash is 2 x 20 min in 2X SSC, 0.05% SDS at 55°C; then 2 x 20 min in 0.1X SSC, 0.1% SDS at 55°C.

Tango 67 is expressed at variable levels in all tissues examined (spleen, thymus, prostate, testes, ovary, small intestine, colon, PBLs, heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas.) The Tango 67 gene is expressed as two transcripts, an ~4.4 kilobase (kb) and ~2.4 kb mRNA, in good agreement with the cDNA clones isolated. The relative levels of the two transcripts vary from tissue to tissue, though with the exception of testes, the 4.4 transcript is significantly more abundant. In the testes the levels of the 4.4 and 2.4 kb mRNAs are approximately the same, and an additional hybridizing transcript is seen at ~800 bp.

Deposit Information

A plasmid containing the human full length nucleotide sequence encoding TANGO 67 was deposited with the American type Culture Collection, Rockville, Maryland, on April 16, 1997 and assigned Accession Number 98410.

The subject culture has been deposited under conditions that assure that access to the culture will be available during the pendency of the patent application to one determined by the Commissioner of Patents and Trademarks to be entitled thereto under 37 CFR 1.14 and 35 USC 122. The deposit is available as required by foreign patent laws in countries wherein counterparts of the subject application, or its progeny, are filed. However, it should be understood that the availability of a deposit does not constitute a license to practice the subject invention in derogation of patent rights granted by governmental action.

Further, the subject culture deposit will be stored and made available to the public in accord with the provisions of the Budapest Treaty for the Deposit of

Microorganisms, i.e., it will be stored with all the care necessary to keep it viable and uncontaminated for a period of at least five years after the most recent request for the furnishing of a sample of the deposit, and in any case, for a period of at least 30 (thirty) years after the date of deposit or for the enforceable life of any patent which can issue disclosing the culture plus five years after the last request for a sample from the deposit. The depositor acknowledges the duty to replace the deposit should the depository be unable to furnish a sample when requested, due to the condition of the deposit. All restrictions on the availability to the public of the subject culture deposit will be irrevocably removed upon the granting of a patent disclosing them.

What is claimed is:

1. An isolated nucleic acid molecule encoding a Tango-67 polypeptide.

2. The nucleic acid molecule of claim 1, said molecule encoding mature human Tango-67.

3. The isolated nucleic acid molecule of claim 1, said molecule comprising a nucleotide sequence encoding a polypeptide having sequence that is at least 85% identical to the sequence of SEQ ID NO:2.

4. The isolated nucleic acid molecule of claim 1, said molecule comprising a nucleotide sequence encoding the amino acid sequence of SEQ ID NO:2.

5. The isolated nucleic acid molecule of claim 1, said molecule comprising the nucleotide sequence of between nucleotide 182 and 850, inclusive, of SEQ ID NO:1.

6. The isolated nucleic acid molecule of claim 1, said molecule hybridizing under stringent conditions to a nucleic acid molecule having the sequence of nucleotides 182 to 850, inclusive, of SEQ ID NO:1 or its complement.

7. A host cell comprising the isolated nucleic acid molecule of claim 1.

8. A nucleic acid vector comprising the nucleic acid molecule of claim 1.

9. The nucleic acid vector of claim 8, wherein said vector is an expression vector.

10. The vector of claim 9, further comprising a regulatory element.

11. The vector of claim 10, wherein the regulatory element is selected from the group consisting of the cytomegalovirus hCMV immediate early gene, the early promoter of SV25 adenovirus, the late promoter of SV25 adenovirus, the lac system, the trp system, the TAC system, the TRC system, the major operator and promoter regions of phage λ, the control regions of fd coat protein, the promoter for 3-phosphoglycerate kinase, the promoters of acid phosphatase, and the promoters of the yeast α-mating factors.

12. The vector of claim 10, wherein said regulatory element directs tissue-specific expression.

13. The vector of claim 9, further comprising a reporter gene.

14. The vector of claim 13, wherein the reporter gene is selected from the group consisting of β-lactamase, chloramphenicol acetyltransferase (CAT), adenosine deaminase (ADA), aminoglycoside phosphotransferase (neo^r, $G403^r$), dihydrofolate reductase (DHFR), hygromycin-B-phosphotransferase (HPH), thymidine kinase (TK), lacZ (encoding β-galactosidase), and xanthine guanine phosphoribosyltransferase (XGPRT).

15. The vector of claim 8, wherein said vector is a plasmid.

16. The vector of claim 8, wherein said vector is a virus.

17. The vector of claim 16, wherein said virus is a retrovirus.

18. A substantially pure Tango-67 polypeptide.

19. The polypeptide of claim 18, said polypeptide being soluble under physiological conditions.

20. The polypeptide of claim 18, said polypeptide comprising an amino acid sequence that is at least 80% identical to the amino acid sequence of SEQ ID NO:2.

21. The polypeptide of claim 18, said polypeptide comprising an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO:2.

22. The polypeptide of claim 18, said polypeptide comprising an amino acid sequence that is identical to the amino acid sequence of SEQ ID NO:2.

23. A substantially pure polypeptide comprising a first portion and a second portion, said first portion comprising a Tango-67 polypeptide and said second portion comprising a detectable marker.

24. An antibody that selectively binds to a Tango-67 polypeptide.

25. The antibody of claim 24, wherein said antibody is a monoclonal antibody.

26. A pharmaceutical composition comprising the polypeptide of claim 18.

27. A method for detecting Tango-67 in a sample, said method comprising:

(a) obtaining a biological sample;

(b) contacting said biological sample with an antibody that specifically binds Tango-67 under conditions that allow the formation of Tango-67-antibody complexes; and

(c) detecting said complexes, if any, as an indication of the presence of Tango-67 in said sample.

28. A method of identifying a compound that modulates the expression of Tango-67, said method comprising comparing the level of expression of Tango-67 in a cell in the presence and absence of a selected compound, wherein a difference in the level of expression in the presence and absence of said selected compound indicates that said selected compound modulates the expression of Tango-67.

29. A method of identifying a compound that modulates the activity of Tango-67, said method comprising comparing the level of activity of Tango-67 in a cell in the presence and absence of a selected compound, wherein a difference in the level of activity in the presence and absence of said selected compound indicates that said selected compound modulates the activity of Tango-67.

30. A method for preparing a medicament for treating a patient suffering from a disorder associated with excessive expression or activity of Tango-67, the method comprising admixing a compound which inhibits expression or activity of Tango-67 with a pharmaceutically acceptable carrier.

31. A method for preparing a medicament for treating a patient suffering from a disorder associated with insufficient expression or activity of Tango-67, the method comprising admixing a compound which increases expression or activity of Tango-67 with a pharmaceutically acceptable carrier.

32. A method for diagnosing a disorder associated with aberrant expression of Tango-67, comprising obtaining a biological sample from a patient and measuring Tango-67 expression in said biological sample, wherein increased or decreased Tango-67 expression in said biological sample compared to a control indicates that said patient suffers from a disorder associated with aberrant expression of Tango-67.

33. A method for diagnosing a disorder associated with aberrant activity of Tango-67, comprising obtaining a biological sample from a patient and measuring Tango-67 activity in said biological sample, wherein increased or decreased Tango-67 activity in said biological sample compared to a control indicates that said patient suffers from a disorder associated with aberrant activity of Tango-67.

34. An isolated nucleic acid molecule which hybridizes to a nucleic acid molecule having the sequence of nucleotides 182 to 850, inclusive, of SEQ ID NO:1 or its complement.

35. An isolated nucleic acid molecule having a sequence which is at least 95% identical to the sequence of nucleotides 182 to 850, inclusive of SEQ ID NO:1.

36. A polypeptide encoded by the nucleic acid molecule of any of claims 34 or 35.

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CCACGCGTCCGCGCGGGCGCTGCGCTGAGGGGACGGCGGGAGGCGCGGCCTGGCCTCGCACTCAAAGCCGCCGCAGCGC   79 SEQ.ID NO: 1

GCCCCGGGCTCGGCCGACCCGGCGGGGATCTAGGGGTGGGCGACTTCGCGGGACCGTGGCGCATGTTTCCTGGGAGTTA  158

                          M  K  L  H  Y  V  A  V  L  T  L  A  I  L    14 SEQ ID NO: 2
CTGATCATCTTCTTTGAAGAAAC ATG AAG TTA CAC TAT GTT GCT GTG CTT ACT CTA GCC ATC CTG  223

 M  F  L  T  W  L  P  E  S  L  S  C  N  K  A  L  C  A  S  D    34
ATG TTC CTG ACA TGG CTT CCA GAA TCA CTG AGC TGT AAC AAA GCA CTC TGT GCT AGT GAT  283

 V  S  K  C  L  I  Q  E  L  C  Q  C  R  P  G  E  G  N  C  S    54
GTG AGC AAA TGC CTC ATT CAG GAG CTC TGC CAG TGC CGG CCG GGA GAA GGC AAT TGC TCC  343

 C  C  K  E  C  M  L  C  L  G  A  L  W  D  E  C  C  D  C  V    74
TGC TGT AAG GAG TGC ATG CTG TGT CTT GGG GCC CTT TGG GAC GAG TGC TGT GAC TGT GTT  403

 G  M  C  N  P  R  N  Y  S  D  T  P  P  T  S  K  S  T  V  E    94
GGT ATG TGT AAT CCT CGA AAT TAT AGT GAC ACA CCT CCA ACT TCA AAG AGC ACA GTG GAG  463

 E  L  H  E  P  I  P  S  L  F  R  A  L  T  E  G  D  T  Q  L   114
GAG CTG CAT GAA CCG ATC CCT TCT CTC TTC CGG GCA CTC ACA GAA GGA GAT ACT CAG TTG  523

 N  W  N  I  V  S  F  P  V  A  E  E  L  S  H  H  E  N  L  V   134
AAT TGG AAC ATC GTT TCT TTC CCT GTT GCA GAA GAA CTT TCA CAT CAT GAG AAT CTG GTT  583

 S  F  L  E  T  V  N  Q  P  H  H  Q  N  V  S  V  P  S  N  N   154
TCA TTT TTA GAA ACT GTG AAC CAG CCA CAC CAC CAG AAT GTG TCT GTC CCC AGC AAT AAT  643

 V  H  A  P  Y  S  S  D  K  E  H  M  C  T  V  V  Y  F  D  D   174
GTT CAC GCG CCT TAT TCC AGT GAC AAA GAA CAC ATG TGT ACT GTG GTT TAT TTT GAT GAC  703

 C  M  S  I  H  Q  C  K  I  S  C  E  S  M  G  A  S  K  Y  R   194
TGC ATG TCC ATA CAT CAG TGT AAA ATA TCC TGT GAG TCC ATG GGA GCA TCC AAA TAT CGC  763

 W  F  H  N  A  C  C  E  C  I  G  P  E  C  I  D  Y  G  S  K   214
TGG TTT CAT AAT GCC TGC TGC GAG TGC ATT GGT CCA GAA TGT ATT GAC TAT GGT AGT AAA  823

 T  V  K  C  M  N  C  M  F  *                                 224
ACT GTC AAA TGT ATG AAC TGC ATG TTT TAA                       853

AGAAGACAAATGCAAACCAAAGCAACTTAGTAAAATAATAGGTATAAAAAGTTATTCTGTAAGTCTGTTGGTTGTATCT  932

TGTATCAGAATCCCAGTAAGTTAAGTTGTAAAGACTTTGGAATAAGTTTCTTTTAAAAATATGACATAGCCAGTGATGT 1011

GTTTAATTATATAACTGTTCTTACTGATTTTATTGCCCCCTAGCAATAAGCCCTTTCCTTTGAATACATGTACAACTTT 1090

GGTCATATGAGAAGCAGGTGCGCAGAGAATTCCTTGAAAGATCTGAGGTTTTTAACATGAAGTCTGATGTGGTTTTCCT 1169

CTAGCATTCCAAAAGGTTTTTGCTTTGAAAGTGTTAGCAGAAGCATGTTGATGTGAATTATGATTTCTTCATGTGCTAC 1248

TGTTAGCACACTGAGTTTTTATAGTTGCACATCATTCCTCATTGTGCCTTGTTTTATCCATTTTATAAATAGAGTAGAT 1327

ATTTGATATACCACTCTGATAACTCATATAAAAATATCATCATAAAAAGCTTAATTTCATCCCTTTTATGTTGGTTTTA 1406

AAAGGTAAATGCTTACCATATTTTATAATTGAGAACTCTTACATAGTAGAATCCATTCTATAATACATGTGTTGACAAA 1485

GCTTTAGAGAAAGTTTCCTATTCTCTTCCATTTCCCCTGCCCAAAGTGCTGACATAGGCAGTGATGAAGAATCTTTACC 1564
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FIGURE (SHEET 1 of 2)

AAGATTTTCAGGGTGTACCTATGAAATTGCTTTAAATGCACTGCTGGTGTAAATAATTAGCAAGCAAAAGCGTTTCTGT 1643

GACTTCAGGTACCAGCTTAAAGAGCACTAGGGATGGGGAACGAATGCCAAATCAGACTCCACCTAGAGCACCAGGAAAC 1722

AGCTTGTACCCTGGTAGGGAAATGGTGTTGCTGAAAGGGGAGGCTGAGCCAGTGCGAGACTGAACTTGTGCAGCCTTAG 1801

CCAAGACAAAGCAGTGTTTTTCAGCAGACGGCTGATGGGACAGGAATTGAAGAAGAGAATTGACTCGTATGAACAGGAC 1880

AGGGTGAAAATGCTGGGAATTATAATGGGAAACAAAACTATCTATGTTCATATTTTGTAATATTTCATTTGTTAAGTTT 1959

ATATCTGGATATAATGTTCTTTTTAAACAAGTATAATCATATCGTCGGAGGTTAAGATTATGAAATTTTAGAATCTCTA 2038

TTCAAGATGATGTTCACTCCAAATACACTACAGAATTTAGTCAACATTTTATATAATGTTTCAATAAATGTTTCTTTCA 2117

ATAAAAAAAAAAAAAAAA 2135

FIGURE (SHEET 2 of 2)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/07603

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.1, 23.5, 24.3, 24.31; 530/399; 435/69.1, 69.4, 71.1, 71.2, 172.3, 325, 252.3, 254.11, 320.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CAS ONLINE, MEDLINE, CAPLUS
search terms: Tango-67, nucleic acid, DNA, polynucleotide, protein, polypeptide, recombinant, cloning

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X, P	WO 97/34998 A1 (HUMAN GENOME SCIENCES, INC.) 25 September 1997 (25.09.97), see entire document, especially pages 44-52.	18-23
A	LEMAIRE et al. Expression Cloning of Siamois, a Xenopus Homeobox Gene Expressed in Dorsal-Vegetal Cells of Blastulae and Able to Induce a Complete Secondary Axis. Cell. 07 April 1995, Vol.81, pages 85-94.	1-23, 26, 34-36
A	JAHN et al. Structures of the Euplotes crassus Tec1 and Tec2 elements: identification of putative transposase coding regions. Gene. 1993, Vol.133, pages 71-78.	1-23, 26, 34-36

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 22 JUNE 1998	Date of mailing of the international search report 19 AUG 1998
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer PREMA MERTZ Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/07603

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MASON et al. Dorsal midline fate in Drosophila embryos requires twisted gastrulation, a gene encoding a secreted protein related to human connective tissue growth factor. Genes and Development. 1994, Vol.8, pages 1489-1501.	1-23, 26, 34-36

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/07603

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-23, 26, 34-36

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/07603

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

C07K 14/475, 14/435, 14/47; C12N 15/12, 15/18, 5/10, 15/63, 15/64, 15/65, 15/66

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

536/23.1, 23.5, 24.3, 24.31; 530/399; 435/69.1, 69.4, 71.1, 71.2, 172.3, 325, 252.3, 254.11, 320.1

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claims 1-23, 26, 34-36, drawn to a nucleic acid molecule encoding Tango-67 polypeptide, a host cell, a nucleic acid vector and a Tango-67 polypeptide.
Group II, claims 24-25, drawn to an antibody that binds Tango-67 polypeptide.
Group III, claim 27, drawn to a method for detecting Tango-67 polypeptide in a sample with an antibody that binds Tango-67 polypeptide.
Group IV, claims 28-29, drawn to a method of identifying a compound that modulates the expression of Tango-67 or a method of identifying a compound that modulates the activity of Tango-67.
Group V, claims 30-31, drawn to a method of treatment of a patient with a compound that inhibits expression or activity of Tango-67 or a compound that increases expression or activity of Tango-67 .
Group VI, claims 32-33, drawn to a method of diagnosing a disorder comprising measuring Tango-67 expression or Tango-67 activity in a biological sample from a patient.

The inventions listed as Groups I-VI do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The inventions listed as Groups I-VI do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:
Pursuant to 37 C.F.R. § 1.475 (d), the ISA/US considers that where multiple products and processes are claimed, the main invention shall consist of the first invention of the category first mentioned in the claims and the first recited invention of each of the other categories related thereto. Accordingly, the main invention (Group I) comprises the first-recited product, a nucleic acid molecule encoding Tango-67 polypeptide, a host cell, a nucleic acid vector and a Tango-67 polypeptide. Further pursuant to 37 C.F.R. § 1.475 (d), the ISA/US considers that any feature which the subsequently recited products and methods share with the main invention does not constitute a special technical feature within the meaning of PCT Rule 13.2 and that each of such products and methods accordingly defines a separate invention.